

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/11, A61K 31/70, C07H 21/00 // C12Q 1/68	A1	(11) International Publication Number: WO 96/23878 (43) International Publication Date: 8 August 1996 (08.08.96)
(21) International Application Number: PCT/US96/01008 (22) International Filing Date: 24 January 1996 (24.01.96) (30) Priority Data: 08/380,650 30 January 1995 (30.01.95) US (71) Applicants (for all designated States except US): HYBRIDON, INC. [US/US]; One Innovation Drive, Worcester, MA 01605 (US). WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH [US/US]; 222 Maple Avenue, Shrewsbury, MA 01545 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): TEMSAMANI, Jamal [MA/US]; 16 Dartmouth Street, Worcester, MA 01604 (US). METELEV, Valeri [RU/US]; Apartment 1, 24 Shrewsbury Green Drive, Shrewsbury, MA 01545 (US). LEVINA, Asya [RU/US]; Apartment G, 24 Shrewsbury Green Drive, Shrewsbury, MA 01545 (US). AGRAWAL, Sudhir [ID/US]; 61 Lamplighter Drive, Shrewsbury, MA 01545 (US). ZAMECNIK, Paul [US/US]; 29 Lebeaux Drive, Shrewsbury, MA 01545 (US). (74) Agents: KERNER, Ann-Louise; Lappin & Kusner, Two Hundred State Street, Boston, MA 02109 (US) et al.		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HUMAN IMMUNODEFICIENCY VIRUS TRANSCRIPTION INHIBITORS AND METHODS OF THEIR USE (57) Abstract Disclosed are methods of inhibiting transcription using a synthetic oligonucleotide complementary to the Watson strand of a double-stranded DNA genome. Also disclosed are synthetic oligonucleotides which specifically inhibit transcription of the HIV-1 genome. Pharmaceutical compositions containing the synthetic oligonucleotides of the invention and methods of treating HIV infection using the oligonucleotides or pharmaceutical compositions of the invention are also provided.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

-1-

**HUMAN IMMUNODEFICIENCY VIRUS TRANSCRIPTION
INHIBITORS AND METHODS OF THEIR USE**

FUNDING

5 This invention was made with Government
support under Grant No. 2 UOL AI-24846-08 awarded
by the National Institutes of Health. The
Government therefore has certain rights in the
invention.

BACKGROUND OF THE INVENTION

10

The present invention relates to the field of
synthetic oligonucleotides which inhibit
transcription. This invention also relates to
human immunodeficiency virus and its treatment
15 using synthetic oligonucleotides which inhibit the
transcription of the viral genome.

Human immunodeficiency virus (HIV) is widely
believed to be the causative agent of acquired
20 immunodeficiency syndrome (AIDS). In recent years
AIDS has become a major worldwide health problem,
and enormous resources have been devoted to caring
for AIDS patients and for HIV-infected individuals
whose disease state has not yet progressed to
25 AIDS. No cure for AIDS or HIV infection has been
identified thus far. After a prolonged
deterioration of health which includes numerous
secondary infections by opportunistic organisms,
virtually all AIDS patients die.

30

A few drugs have been developed which may
slow the progression of HIV infection to AIDS.

-2-

These drugs generally inhibit the synthesis of HIV DNA, for example, by incorporating nucleotide analogs which prevent further nucleotide addition such as azidothymidine, dideoxycytidine, dideoxyinosine, and the like into viral DNA. However, these agents are not specific for viral DNA and thus the analogs are also randomly incorporated into the infected individual's DNA, causing significant damage to the infected individual as well as to the HIV. Recent studies have cast doubt on the long-term efficacy of azidothymidine therapy. Moreover, the nucleotide analogs also have severe side effects, and an analog may lose its efficacy against HIV in a particular individual, for unknown reasons. In these cases, the individuals may be switched to a new analog, or to a combination of analogs. Some individuals cannot tolerate a particular nucleotide analog, and some individuals cannot tolerate nucleotide analog therapy at all. At this time it is not possible to predict how an HIV-infected individual will respond to nucleotide analog therapy prior to actually administering the drugs.

In an effort to improve the specificity of anti-HIV therapy, therapeutic agents have been developed which specifically bind to HIV mRNA and thereby inhibit translation of viral proteins. A number of such therapeutic agents, generally known as antisense agents, are described, for example, in U.S. Patent No. 4,806,463 and PCT publication No. WO 94/08004.

-3-

Another anti-HIV strategy employs oligonucleotides which bind to double-stranded DNA at regions of high homopyrimidine content, to form triple helical structures which are believed to block transcription and/or replication. This approach, known as the antigene strategy, is limited by its requirement for regions of high homopyrimidine content at a therapeutically relevant site within the viral DNA. Moreover, the nature of the hydrogen bonds which form the triple helical structures required for action of such agents is inherently weaker than that of the hydrogen bonds which form double helical structures. Thus antigene agents are expected to have less efficacy than antisense agents.

HIV is well-known to mutate extensively, resulting in a large number of strains which have been isolated from clinical specimens. New strains of HIV are likely to develop as the worldwide epidemic progresses. Moreover, infected individuals may respond idiosyncratically to known HIV therapeutic agents. Thus, there is a continued need for development of additional anti-HIV therapeutic agents.

SUMMARY OF THE INVENTION

It has been discovered that a synthetic oligonucleotide, preferably not bound to an intercalating agent, can be used to inhibit transcription. This discovery has been exploited to develop the present invention which includes a method of inhibiting the transcription of a gene.

-4-

In this method the transcription initiation region of the Watson strand of the gene from which transcription is to be inhibited is contacted with a synthetic oligonucleotide. As defined herein, the "Watson" strand of an HIV gene is the non-coding or antisense strand of the gene, while the "Crick" strand of an HIV gene is the coding or sense strand of the gene. The term "transcription initiation region" refers herein to the site at which the transcription of the genomic DNA into RNA begins. The oligonucleotide has a first nucleotide sequence complementary to and hybridizable with a second nucleotide sequence within the transcription initiation region, and in preferred embodiments, is not linked to an intercalating agent.

A number of short synthetic oligonucleotides have been identified which specifically inhibit transcription of the HIV genome, and thus which are useful as anti-HIV therapeutic agents. These oligonucleotides of the invention correspond to nucleotide sequences found within the transcription initiation region of the Crick strand of the HIV genome.

In one embodiment, the invention provides a synthetic oligonucleotide comprising a first nucleotide sequence complementary to and hybridizable with a second nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome.

-5-

In some embodiments, the synthetic oligonucleotide is from about nine to about twenty-seven nucleotides in length. In other embodiments, the oligonucleotide is from about
5 nine to about fifteen nucleotides in length.

In yet another embodiment, the invention provides a method of inhibiting the expression of HIV genes including *gag*, *rev*, *tat*, *nef*, or *pol* genes,
10 comprising the step of contacting the Watson strand of the HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a nucleotide sequence contained within the transcription initiation region of the Watson
15 strand of the HIV genome.

The present invention also encompasses pharmaceutical compositions containing the synthetic oligonucleotides described herein, and
20 methods of treatment or use which employ the synthetic oligonucleotides described herein.

-6-

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1A is a diagrammatic representation of the HIV-1 genome;

FIG. 1B is a schematic representation of the transcription initiation region of the HIV-1 genome;

FIG. 2A is a schematic representation of the sequence of the T7 promoter in plasmid pDAB72 and the transcription initiation site, wherein the arrow indicates the direction of transcription;

FIG. 2B is an autoradiogram of a gel demonstrating the inhibition of T7 RNA polymerase transcription of the HIV *gag* gene sequence contained in plasmid pDAB72 by the synthetic oligonucleotide of SEQ ID NO:13. The oligonucleotides were annealed to linearized plasmid prior to transcription. Lane 1, no oligonucleotide control; lane 2, sense oligonucleotide having SEQ ID NO:13; lane 3, antisense oligonucleotide having SEQ ID NO:14; lane 4, random oligonucleotide having SEQ ID NO:15;

-7-

FIG. 3 is an autoradiogram of a gel demonstrating that the synthetic oligonucleotide with SEQ ID NO:1 inhibited T7 RNA polymerase transcription of the HIV *gag* gene sequence contained in plasmid pDAB72 without a pre-annealing step. Lane 1, sense oligonucleotide having SEQ ID NO:13; lane 2, antisense oligonucleotide having SEQ ID NO:14; lane 3, random oligonucleotide having SEQ ID NO:15;

FIG. 4 is an autoradiogram of a gel demonstrating that inhibition of T7 RNA polymerase transcription by the oligonucleotide of SEQ ID NO:13 is specific for HIV *gag* gene sequences.

Lane 1, no oligonucleotide control; lane 2, sense oligonucleotide having SEQ ID NO:13; lane 3, antisense oligonucleotide having SEQ ID NO:14; lane 4, random oligonucleotide having SEQ ID NO:15;

FIG. 5 is an autoradiogram of a gel showing that inhibition of T7 RNA polymerase transcription of the HIV *gag* gene sequences contained in plasmid pDAB72 by an oligonucleotide of the invention is concentration dependent. Lane 1, no oligonucleotide control; lanes 2, 5, 8, sense oligonucleotide having SEQ ID NO:13; lanes 3, 6, 9, antisense oligonucleotide having SEQ ID NO:14; lanes 4, 7, 10, random oligonucleotide having SEQ ID NO:15;

-8-

FIG. 6A is a schematic representation of the sequence of the transcription initiation site and the SP6 promoter in the pGEM-*gag* plasmid, wherein the arrow indicates the direction of transcription;

5

FIG. 6B is an autoradiogram of a gel showing the inhibition of SP6 RNA polymerase transcription of the HIV *gag* gene sequences contained in plasmid pGEM-*gag* by an oligonucleotide of the invention. Lane 1, no oligonucleotide control; lane 2, sense oligonucleotide having SEQ ID NO:16; lane 3, antisense oligonucleotide having SEQ ID NO:17; lane 4, random oligonucleotide having SEQ ID NO:15; and

10

15

FIG. 7 is an autoradiogram of a gel showing inhibition of T7 RNA polymerase transcription of the HIV *gag* gene sequences contained in plasmid pDAB72 by oligonucleotides of the invention. Lane 1, no oligonucleotide control; lane 2, sense oligonucleotide having SEQ ID NO:13; lane 3, antisense oligonucleotide having SEQ ID NO:14; lane 4, sense oligonucleotide having SEQ ID NO:18; lane 5, antisense oligonucleotide having SEQ ID NO:19; and lane 6, random oligonucleotide having SEQ ID NO:15.

20

25

-9-

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patents, allowed applications, and references cited herein are hereby incorporated by reference.

10 In accordance with the present invention, transcription of a gene may be inhibited by contacting the transcription initiation region of the Watson strand of gene with a synthetic oligonucleotide including a first nucleotide
15 sequence complementary to and hybridizable with a second nucleotide sequence contained within the transcription initiation region of the Watson strand of the gene.

20 As such, this method is useful for examining the function of various genes, including those essential to animal development, for example, in an *in vitro* system. Presently, gene function can only be examined by the arduous task of making a
25 "knock out" animal such as a mouse. This task is difficult, time-consuming and cannot be accomplished for genes essential to animal development since the "knock out" would produce a lethal phenotype. The present invention overcomes
30 the shortcomings of this model.

 The invention also provides synthetic oligonucleotides which inhibit the transcription of any HIV gene. The oligonucleotides of the

-10-

invention comprise a first nucleotide sequence complementary to and hybridizable with a second nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome. In the case of HIV-1, the transcription initiation region begins at nucleotide +1 of the long terminal repeat (LTR) region of the 5' end of the proviral DNA (see FIG. 1A). FIG. 1B shows the sequence of the transcription initiation site of HIV-1 in more detail. The region immediately preceding the transcription initiation region is the promoter region to which RNA polymerase initially and specifically binds. Oligonucleotides of the invention should include nucleotides which are complementary to at least one of the first 10 nucleotides (+1---+10) of the Watson strand of the transcription initiation region. Any one of, or any combination of, the oligonucleotides of the invention (examples of which are set forth in Table 1 below may be used to inhibit the transcription of the HIV *gag*, *rev*, *tat*, *nef*, *pol*, or any other HIV gene.

25

TABLE 1

5	Targeted Site on HIV genome	Length (Nucleotides)	Sequence (5' to 3')	SEQ ID NO:
10	+1 ---- +9	9	GGTCTCTCT	1
	+4 ---- +12	9	CTCTCTGGT	2
	+9 ---- +17	9	TGGTTAGAC	3
	+1 ---- +14	14	GGTCTCTCTGGTTA	4
	+5 ---- +18	14	TCTCTGGTTAGACC	5
	+10 --- +23	14	GGTTAGACCAGATC	6
	+1 ---- +20	20	GGTCTCTCTGGTTAGACCAG	7
	+6 ---- +26	20	CTCTGGTTAGACCAGATCTG	8
	+8 ---- +28	20	CTGGTTAGACCAGATCTGAG	9
	+1 ---- +27	27	GGTCTCTCTGGTTAGACCAGATCTGAG	10
15	+3 ---- +29	27	TCTCTCTGGTTAGACCAGATCTGAGCC	11
	+7 ---- +33	27	TCTGGTTAGACCAGATCTGAGCCTGGG	12
	+5 ---- +18	14	UCUCUGGUAGAGACC	20

-12-

Without being bound by any mechanistic theory, it is believed that the oligonucleotides of the present invention act as follows. After HIV-1 infection of a host cell, the viral RNA genome, which contains the Crick or coding strands of the viral genes, is reverse transcribed into a double-stranded DNA provirus. The HIV-1 provirus is integrated into the DNA of the host cell, and new viral RNA genomes are transcribed from the Watson or non-coding strand of the provirus by DNA-dependent RNA polymerase II of the host cell. The DNA-dependent RNA polymerase II is believed to act by recognizing the promoter sequence upstream from the long terminal repeat (LTR) region (see FIGS. 1A and 1B) adjacent to the *gag* gene, and binding to the DNA helix in its "closed" or hydrogen bonded form. A short section (12-15 nucleotides) of the DNA helix is then unwound, forming an "open" complex or "transcription eye" of non-hydrogen bonded DNA strands. The DNA-dependent RNA polymerase II then catalyzes polymerization of RNA into a single pre-mRNA transcript encoding all of the HIV-1 genes using the Watson or non-coding strand of the DNA as a template. This transcript is then spliced into multiple mRNAs encoding the HIV genes. The oligonucleotides of the invention are believed to act as an inhibitor of the DNA-dependent RNA polymerase II within the open or unwound region of the promoter of the HIV-1 genome. As such, a single oligonucleotide of the invention can inhibit transcription of all of the HIV-1 genes normally transcribed during infection.

-13-

The oligonucleotides of the invention are short synthetic oligonucleotides which, as used herein, encompass chemically synthesized polymers of about nine to about twenty-seven, preferably from about nine to about fifteen, and most preferably from about nine to fourteen nucleotide monomers (nucleotide bases) connected together or linked by at least one 5' to 3' internucleotide linkage.

10

The oligonucleotides of the invention are composed of deoxyribonucleotides, ribonucleotides, or any combination thereof. Thus, the representative oligodeoxyribonucleotides of the invention set forth in Table 1 may also be oligoribonucleotide or chimeric oligonucleotides. The 5' end of one nucleotide and the 3' end of another nucleotide are covalently linked, in some cases, via a phosphodiester internucleotide linkage. These oligonucleotides can be prepared by art recognized methods such as phosphoramidate, H-phosphonate chemistry, or methylphosphoramidate chemistry (see, e.g., Uhlmann et al. (1990) *Chem. Rev.* 90:543-584; Agrawal (ed.) *Meth. Mol. Biol.*, Humana Press, Totowa, NJ (1993) Vol. 20; and U.S. Patent 5,149,798) which can be carried out manually or by an automated synthesizer and then processed (reviewed in Agrawal et al. (1992) *Trends Biotechnol.* 10:152-158).

30

The oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to nucleotide sequences contained within the

-14-

transcription initiation region of the Watson strand of the HIV genome.

5 The term "modified oligonucleotide" as used
herein describes an oligonucleotide in which at
least two of its nucleotides are covalently linked
via a synthetic linkage, i.e., a linkage other
than a phosphodiester linkage between the 5' end
10 of one nucleotide and the 3' end of another
nucleotide in which the 5' nucleotide phosphate
has been replaced with any number of chemical
groups.

15 Preferable synthetic linkages include
alkylphosphonates, phosphorothioates,
phosphorodithioates, phosphate esters,
alkylphosphonothioates, phosphoramidates,
carbamates, carbonates, phosphate triesters,
acetamidate, and carboxymethyl esters.
20 Oligonucleotides with these linkages or other
modifications can be prepared according to known
methods (see, e.g., Agrawal and Goodchild
(*Tetrahedron Lett.* (1987) 28:3539-3542); Agrawal et al.
(*Proc. Natl. Acad. Sci. (USA)* (1988) 85:7079-7083);
25 Uhlmann et al. *Chem. Rev.* (1990) 90:534-583; Agrawal
et al. (*Trends Biotechnol.* (1992) 10:152-158; Agrawal
(ed.) *Meth. Mol. Biol.*, Humana Press, Totowa, NJ
(1993) Vol. 20).

30 The term "modified oligonucleotide" also
encompasses oligonucleotides with a modified base
and/or sugar. For example, modified
oligonucleotides, include a 3', 5'-substituted

-15-

oligonucleotide having at both its most 3' and 5' positions sugars which are attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position). Such a modified oligonucleotide may also be referred to as a capped species. Other modified ribonucleotide-containing oligonucleotides include a 2'-O-alkylated ribose groups such as a 2'-O-methylated ribose, or oligonucleotides with arabinose instead of ribose. In addition, unoxidized or partially oxidized oligonucleotides having a substitution in one nonbridging oxygen per nucleotide in the molecule are also considered to be modified oligonucleotides.

Such modifications can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule (reviewed in Agrawal et al. (1992) *Trends Biotechnol.* 10:152-158 and Agrawal (ed.) *Meth. Mol. Biol.* (Humana Press, Totowa, NJ (1993) Vol. 20). Also considered as modified oligonucleotides are oligonucleotides having nuclease resistance-conferring bulky substituents at their 3' and/or 5' end(s) and/or various other structural modifications not found *in vivo* without human intervention. Other modifications include those which are internal or are at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose

-16-

and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the viral genome.

5

The synthetic oligonucleotides of the invention may be used to identify the presence of HIV nucleic acids in cells *in vitro*, for example, by labelling the oligonucleotide and screening for double-stranded, labelled DNA in the cells by *in situ* hybridization or some other art-recognized detection method.

The oligonucleotides of the invention may also be used to inhibit transcription of HIV genes within infected host cells and thus to inhibit production of HIV virions by those cells. The synthetic oligonucleotides of the invention are thus useful for treatment of HIV infection and AIDS in mammals, particularly the treatment of mammals used as animal models to study HIV infection and AIDS. The synthetic oligonucleotides of the invention are also useful for treatment of humans infected with HIV and those suffering from AIDS.

The synthetic oligonucleotides of the invention may be used as part of a pharmaceutical composition when combined with a physiologically and/or pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The term "physiologically

-17-

acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Such a composition may contain, in addition to the synthetic oligonucleotide and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance inhibition of HIV virion production by infected cells. For example, combinations of synthetic oligonucleotides, each of which inhibits transcription of a different HIV gene, may be used in the pharmaceutical compositions of the invention. The pharmaceutical composition of the invention may further contain nucleotide analogs such as azidothymidine, dideoxycytidine, dideoxyinosine, and the like. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with the synthetic oligonucleotide of the invention, or to minimize side-effects caused by the synthetic oligonucleotide of the invention. Conversely, the synthetic oligonucleotide of the invention may be included in formulations of a particular anti-HIV factor and/or agent to minimize side effects of the anti-HIV factor and/or agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which the synthetic oligonucleotides of the

-18-

invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323.

The pharmaceutical composition of the invention may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells, as described by Zhao et al. (in press).

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., healing of chronic conditions characterized by HIV infection or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect,

-19-

whether administered in combination, serially or simultaneously.

5 In practicing the method of treatment or use
of the present invention, a therapeutically
effective amount of one or more of the synthetic
oligonucleotide of the invention is administered
to a mammal infected with HIV. The synthetic
oligonucleotide of the invention may be
10 administered in accordance with the method of the
invention either alone or in combination with
other therapies such as treatments employing
cytokines, lymphokines, other hematopoietic
factors, other anti-viral agents, and the like.
15 When co-administered with one or more cytokines,
lymphokines or other hematopoietic factors, other
anti-viral agents, the synthetic oligonucleotide
of the invention may be administered either
simultaneously with the cytokine(s),
20 lymphokine(s), other hematopoietic factor(s),
other antiviral agents, and the like, or
sequentially. If administered sequentially, the
attending physician will decide on the appropriate
sequence of administering the synthetic
25 oligonucleotide of the invention in combination
with cytokine(s), lymphokine(s), other
hematopoietic factor(s), anti-viral agents, and
the like.

30 Administration of the synthetic
oligonucleotide of the invention used in the
pharmaceutical composition or to practice the
method of the present invention can be carried out
in a variety of conventional ways, such as oral

-20-

ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered orally, the synthetic oligonucleotide will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide and preferably from about 1 to 50% synthetic oligonucleotide.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered by intravenous, cutaneous or subcutaneous injection, the synthetic oligonucleotide will be in the form of a pyrogen-free, parenterally acceptable aqueous solution.

-21-

The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to the synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention

-22-

should contain about 1.0 μ g to about 100 mg of synthetic oligonucleotide per kg body weight.

5 The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the synthetic oligonucleotide will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

20 The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be used to obtain similar results.

EXAMPLE 1

25 Oligonucleotide Synthesis

30 Oligodeoxynucleotides were synthesized on an automated synthesizer (Biosearch 8700; Milligen) as described by Beaucage, (*Protocols for Oligonucleotides and Analogs: Synthesis and Properties*, (Agrawal, ed.) Humana Press, Totowa, N.J. (1993) Vol. 20 pp. 33-61). After full deprotection, oligodeoxynucleotides were purified by ion-exchange HPLC on a Whatman PartiSphere SAX (Whatman, Clifton, NJ) cartridge

-23-

and finally desalted on Waters Sep-Pak C18 cartridges (Waters, Milford, MA).

5 Using these methods, the oligonucleotides of
SEQ ID NO:13, SEQ ID NO:16, and SEQ NO:18 were
prepared. The synthetic oligonucleotide of SEQ ID
NO:13 is a 14mer corresponding to nucleotides +4
to +17 of the plasmid pDAB72 containing the Watson
strand of the HIV *gag* gene. The synthetic
10 oligonucleotide of SEQ ID NO:16 is a 14mer
corresponding to nucleotides +1 to +14 of the
pGEM-*gag* plasmid containing the Watson strand of
the HIV *gag* gene. The synthetic oligonucleotide
of SEQ ID NO:18 is a 9mer corresponding to
15 nucleotides 7 to 15 of the plasmid pDAB72
containing the Watson strand of the HIV *gag* gene.
Additional oligonucleotides were synthesized
having SEQ ID NOS:14, 17, and 19 which are
complementary to the oligonucleotides of SEQ ID
20 NO:13, SEQ ID NO:16, and SEQ ID NO:18,
respectively, representing corresponding regions
of the Crick or sense strand of the HIV *gag* gene
in the plasmid. These oligonucleotides were used
as oligonucleotide controls in the transcription
25 experiments set forth in Example 2.

EXAMPLE 2

Transcription Inhibition

30 A. Plasmids

The plasmid pDAB72 (Erikson-Viitanen et al.
(1989) *AIDS Res. Hum. Retroviruses* 5:577-591) represents

-24-

the NcoI-BamHI insert, containing HIV sequences in the pTZ19R vector (Erikson-Viitanen et al., (*ibid.*)). Linearization of this plasmid by PstI and transcription by T7 RNA polymerase yields a
5 *gag* RNA of about 640 nucleotides. The plasmid pGEM-*gag* was made by inserting the fragment SacI-ApaI (nucleotides 224-1551) of HIV in the SacI-ApaI sites of pGEM-11z(+) (Promega, Madison, WI). Linearization of pGEM-*gag* by SacI and
10 transcription by SP6 RNA polymerase yields a RNA of about 1360 nucleotides.. The plasmid pGU2^{pre} contains the sequence of U2 RNA (Kleinschmidt et al. (1989) *Nucleic Acids Res.* 17:4817-4828; Temsamani et al. (1991) *Antisense Res. & Dev.* 4: 35-42).
15 Linearization of pGU2PRE by BamHI and transcription by T7 RNA polymerase yields an RNA of about 200 nucleotides.

B. *In vitro* Transcription

20

Each transcription reaction (15 μ l) contained 100 ng of the linearized plasmid, 40 mM Tris-HCl pH 7.5, 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl, 20 Units of RNasin, (Promega, Madison, WI), 2 mM
25 DTT, 0.5 mM of ATP, CTP, UTP and 0.05 mM GTP, and 10 μ Ci of ³²P-GTP (specific activity 400 Ci/mmol, Amersham), the oligonucleotide (10 μ M) and 10 Units of DNA-dependent T7 RNA polymerase (Promega, Madison, WI). The reaction was carried out at
30 37°C for 20 minutes. The products were visualized by autoradiography after electrophoresis through a 4% polyacrylamide gel containing 90 mM Tris-borate pH 8.3/8.3 M urea / 2.5 mM EDTA. The gel was

-25-

exposed for autoradiography and the results analyzed using the Molecular Imager GS-250 (Bio-Rad, Hercules, CA).

5 Initially, oligonucleotides were annealed to the linearized plasmid prior to addition of T7 RNA polymerase. When the sythetic oligonucleotide of SEQ ID NO:13 was annealed to the linearized pDAB72 plasmid (see FIG. 2A), the plasmid was first added
10 to the oligonucleotide in 5 μ l of transcription buffer (40 mM Tris-HCl pH 7.5, 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl). The sample was heated at 90°C for 5 minutes then cooled down at room temperature for 30 minutes.

15 The transcription reaction was then carried out for 20 minutes at 37°C, and the products were analyzed by gel electrophoresis. As judged by the migration on the gel, the expected HIV *gag* RNA of
20 640 nucleotides was transcribed in the absence of any added oligonucleotide (FIG. 2B, lane 1). An additional band was observed in all lanes which is believed to correspond to a transcription arrest of about 620 nucleotides. As measured by
25 densitometry, the synthetic oligonucleotide of SEQ ID NO:13 inhibited transcription by more than 80% (lane 2 of FIG. 2B), compared to the inhibition measured in the "no oligonucleotide" control (lane 1 of FIG. 2B). The oligonucleotide having SEQ ID
30 NO:14, which is the Watson complement of SEQ ID NO:13 (lane 3 of FIG. 2B) inhibited transcription by about 15%, as did a random 14mer oligonucleotide having SEQ ID NO:16 (lane 4 of FIG. 2B) When the transcription reaction was

-26-

carried out for different time points (10 minutes and 45 minutes), similar results were obtained. The inhibition observed with the random oligonucleotide may be a non-specific effect.

5

FIG. 3 shows that when the synthetic oligonucleotide of SEQ ID NO:13 was incubated with the plasmid DNA without pre-annealing, a similar level of inhibition was observed (lane 1).

10

Results similar to those of FIG. 2A and 2B were also obtained for the oligonucleotide having SEQ ID NO:14, which is the Watson complement of the synthetic oligonucleotide of SEQ ID NO:13 (lane 2 of FIG. 2), and for the random 14mer

15

oligonucleotide having SEQ ID NO:15 (lane 3 of FIG. 3). These results indicate that the synthetic oligonucleotide of SEQ ID NO:13 binds specifically to the DNA open complex.

20

To confirm that the synthetic oligonucleotide of SEQ ID NO:13 specifically inhibits transcription of HIV *gag* RNA, transcription of U2 RNA from the pGU2^{pre} plasmid was measured in the absence of added oligonucleotide (lane 1 of FIG.

25

4) and in the presence of the synthetic oligonucleotide of SEQ ID NO:13 (lane 2 of FIG. 4), the Watson complement of the synthetic oligonucleotide of SEQ ID NO:13 having SEQ ID NO:14 (lane 3 of FIG. 4), and the random 14mer

30

oligonucleotide having SEQ ID NO:15 (lane 4 of FIG. 4). Some non-specific inhibition of U2 RNA transcription was observed for all nucleotides added to the pGU2^{pre} plasmid. However, FIG. 4 shows that the synthetic oligonucleotide of SEQ ID

-27-

NO:13, which was very efficient in inhibiting HIV *gag* transcription, as indicated in FIGS. 2A, 2B, and 3, did not inhibit transcription of U2 RNA, further indicating the specificity of HIV *gag* transcription inhibition by the synthetic oligonucleotide of SEQ ID NO:13.

The concentration dependence of the inhibition of HIV *gag* transcription by the synthetic oligonucleotide of SEQ ID NO:13 is shown in FIG. 5. Plasmid pDAB72 was incubated with 2, 5, and 10 μ M oligonucleotide of SEQ ID NO:13 (lanes 2, 5, and 8, respectively), 2, 5, and 10 μ M of the Watson complement of the synthetic oligonucleotide of SEQ ID NO:13 having SEQ ID NO:14 (lanes 3, 6, and 9), and the random 14mer oligonucleotide having SEQ ID NO:15 (lanes 4, 7, and 10). At 2 μ M, almost no inhibition of transcription was observed. At 5 μ M, the synthetic oligonucleotide of SEQ ID NO:13 inhibited HIV *gag* transcription by about 40% as compared with the no oligonucleotide control (lane 1). At 10 μ M the synthetic oligonucleotide of SEQ ID NO:13 demonstrated similar levels of HIV *gag* transcription inhibition as were observed in FIGS. 2A, 2B, and 3. No significant inhibition of HIV *gag* transcription was observed with the Watson complement of the synthetic oligonucleotide of SEQ ID NO:13 having SEQ ID NO:14, or with the random 14mer oligonucleotide having SEQ ID NO:15. When 20 μ M of each oligonucleotide was added to plasmid pDAB72, non-specific inhibition of transcription was observed.

-28-

EXAMPLE 3

Other Oligonucleotides

To confirm that inhibition of HIV *gag* transcription by synthetic oligonucleotides complementary to the transcription initiation region of the HIV genome is not limited to T7 RNA polymerase, transcription experiments were performed using the SP6 RNA polymerase, the pGEM-*gag* plasmid, and the synthetic oligonucleotide of SEQ ID NO:16, which is complementary to nucleotides at positions +1 to +14 of the plasmid. FIG. 6A shows the position of the transcription site starting at +1. The HIV *gag* transcription inhibiting activity of the synthetic oligonucleotide of SEQ ID NO:16 is demonstrated in FIG. 6B, lane 2, as compared to the "no oligonucleotide" control in lane 1. Using the synthetic oligonucleotide of SEQ ID NO:16, about 80% inhibition is found. No significant inhibition of HIV *gag* transcription was observed with the Watson complement of the synthetic oligonucleotide of SEQ ID NO:14 having SEQ ID NO:17 (lane 3) or with the random 14mer oligonucleotide having SEQ ID NO:15 (lane 4).

To determine whether the length of the oligonucleotide effected its ability to inhibit transcription, the 9mer synthetic oligonucleotide of SEQ ID NO:18 was compared to the 14mer synthetic oligonucleotide of SEQ ID NO:13 in the T7 transcription system described above. FIG. 7 shows that the shorter (9mer) oligonucleotide

-29-

(lane 4) inhibited transcription of the HIV *gag* gene as efficiently as did the longer (14mer) oligonucleotide (lane 2). Inhibition of HIV *gag* transcription by the synthetic oligonucleotide of SEQ ID NO:18 was specific, as indicated by the lack of inhibition by the Watson complement of the synthetic oligonucleotide of SEQ ID NO:18 having SEQ ID NO:19 (lane 5) and by the random 14mer oligonucleotide of SEQ ID NO:15 (lane 6). FIG. 7 further shows the lack of inhibition with the Watson complement of the synthetic oligonucleotide of SEQ ID NO:13 having SEQ ID NO:14 (lane 3).

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

-30-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Hybridon, Inc.
Worcester Foundation for
Experimental Biology
- (ii) TITLE OF INVENTION: Human Immunodeficiency Virus
Transcription Inhibitors and Methods of Their Use
- (iii) NUMBER OF SEQUENCES: 20
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Lappin & Kusmer
 - (B) STREET: 200 State Street
 - (C) CITY: Boston
 - (D) STATE: Massachusetts
 - (E) COUNTRY: USA
 - (F) ZIP: 02109
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE:
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Kerner, Ann-Louise
 - (B) REGISTRATION NUMBER: 33,523
 - (C) REFERENCE/DOCKET NUMBER: HYZ-037PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 617-330-1300
 - (B) TELEFAX: 617-330-1311

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

-31-

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTCTCTCT

9

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTCTCTGGT

9

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGTTAGAC

9

(2) INFORMATION FOR SEQ ID NO:4:

-32-

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA or cDNA/RNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGTCTCTCTG GTTA

14

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA or cDNA/RNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTCTGGTTA GACC

14

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA or cDNA/RNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

-33-

GGTTAGACCA GATC

14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGTCTCTCTG GTTAGACCAG

20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTCTGGTTAG ACCAGATCTG

20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

-34-

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGTTAGAC CAGATCTGAG

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGTCTCTCTG GTTAGACCAG ATCTGAG

27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCTCTCTGGT TAGACCAGAT CTGAGCC

27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

-35-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA or cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCTGGTTAGA CCAGATCTGA GCCTGGG

27

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGCTTAAAC CATG

14

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CATGGTTTAA GCTT

14

-36-

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AACCGGAATT CGAT

14

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAATACTCAA GCTT

14

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

-37-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGCTTGAGT ATTC

14

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTTAAACCA

9

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAATTTAAG

9

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-38-

- (ii) MOLECULE TYPE: RNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

UCUCUGGUUA GACC

14

-39-

What is claimed is:

1. A synthetic oligonucleotide comprising a
first nucleotide sequence complementary to and
5 hybridizable with a second nucleotide sequence
contained within the transcription initiation
region of the Watson strand of the HIV genome, the
second nucleotide sequence being a single-stranded
nucleic acid.
- 10 2. The oligonucleotide of claim 1 wherein the
oligonucleotide comprises from about nine to about
twenty-seven nucleotides.
- 15 3. The oligonucleotide of claim 2, wherein the
oligonucleotide comprises from about nine to about
fifteen nucleotides.
- 20 4. The oligonucleotide of claim 1 which is
modified.
5. A pharmaceutical composition comprising the
synthetic oligonucleotide of claim 1.

25

-40-

6. A method of inhibiting the transcription of a gene comprising the step of contacting the transcription initiation region of the Watson strand of the gene with a synthetic
5 oligonucleotide having a first nucleotide sequence complementary to and hybridizable with a second nucleotide sequence within the transcription initiation region, the second nucleotide sequence being a single-stranded nucleic acid, and the
10 oligonucleotide not being linked to an intercalating agent.
7. A method of inhibiting the expression of the HIV *gag* gene comprising the step of contacting the
15 HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome, the second nucleotide
20 sequence being a single-stranded nucleic acid.
8. The method of claim 7 wherein the oligonucleotide comprises from about nine to about twenty-seven nucleotides.
25
9. The method of claim 8 wherein the oligonucleotide comprises from about nine to about fifteen oligonucleotides.
- 30 10. The method of claim 7 wherein the oligonucleotide is modified.

-41-

11. A method of inhibiting the expression of the HIV *rev* gene comprising the step of contacting the HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a
5 nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome, the second nucleotide sequence being a single-stranded nucleic acid.
- 10 12. The method of claim 11 wherein the oligonucleotide comprises from about nine to about twenty-seven nucleotides.
- 15 13. The method of claim 12 wherein the oligonucleotide comprises from about nine to about fifteen oligonucleotides.
- 20 14. The method of claim 11 wherein the oligonucleotide is modified.
- 25 15. A method of inhibiting the expression of the HIV *tat* gene comprising the step of contacting the HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a
nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome, the second nucleotide sequence being a single-stranded nucleic acid.
- 30 16. The method of claim 15 wherein the oligonucleotide comprises from about nine to about twenty-seven nucleotides.

-42-

17. The method of claim 16 wherein the oligonucleotide comprises from about nine to about fifteen oligonucleotides.
- 5 18. The method of claim 15 wherein the oligonucleotide is modified.
- 10 19. A method of inhibiting the expression of the HIV *pol* gene comprising the step of contacting the HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome, the second nucleotide
15 sequence being a single-stranded nucleic acid.
- 20 20. The method of claim 19 wherein the oligonucleotide comprises from about nine to about twenty-seven nucleotides.
21. The method of claim 20 wherein the oligonucleotide comprises from about nine to about fifteen oligonucleotides.
- 25 22. The method of claim 19 wherein the oligonucleotide is modified.

-43-

23. A method of inhibiting the expression of the HIV *nef* gene comprising the step of contacting the HIV genome with a synthetic oligonucleotide complementary to and hybridizable with a nucleotide sequence contained within the transcription initiation region of the Watson strand of the HIV genome, the second nucleotide sequence being a single-stranded nucleic acid.
24. The method of claim 23 wherein the oligonucleotide comprises from about nine to about twenty-seven nucleotides.
25. The method of claim 24 wherein the oligonucleotide comprises from about nine to about fifteen oligonucleotides.
26. The method of claim 23 wherein the oligonucleotide is modified.

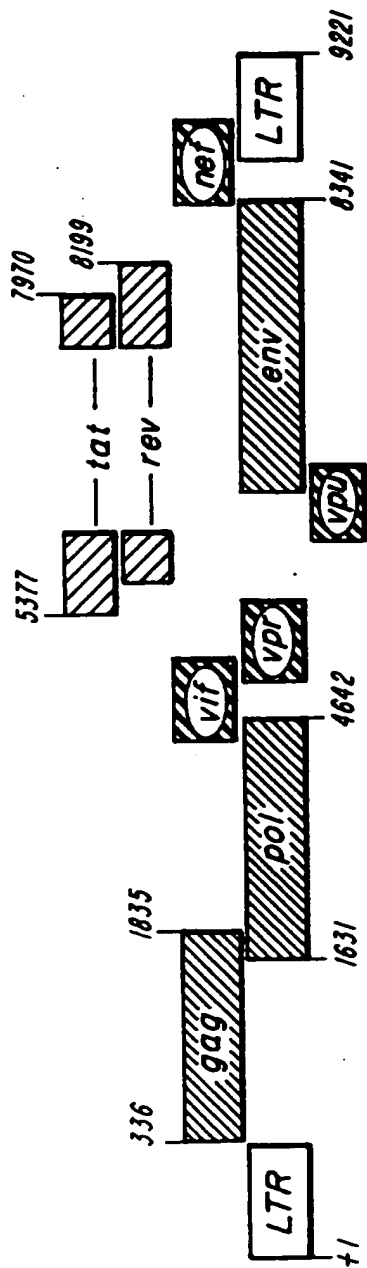
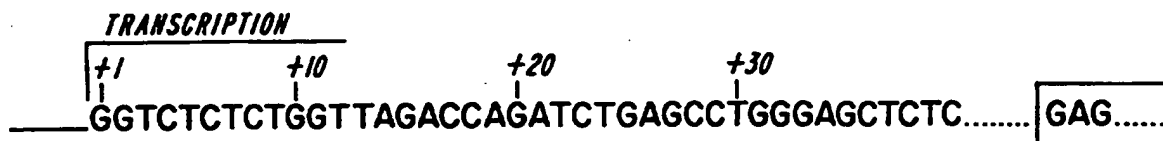
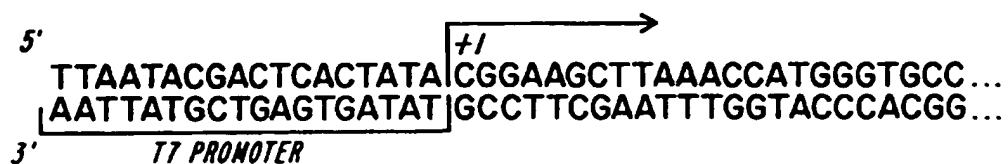
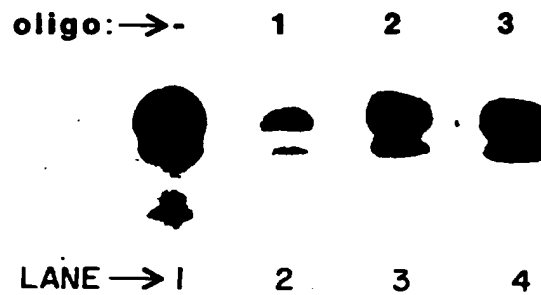
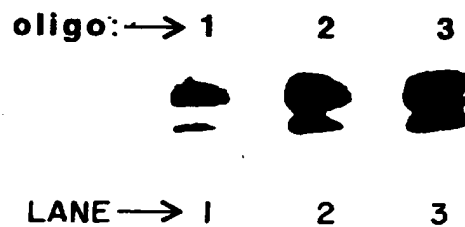


FIG. 1A

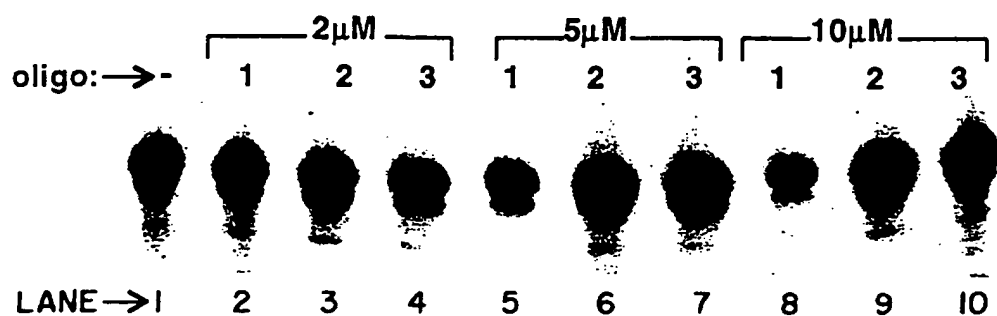
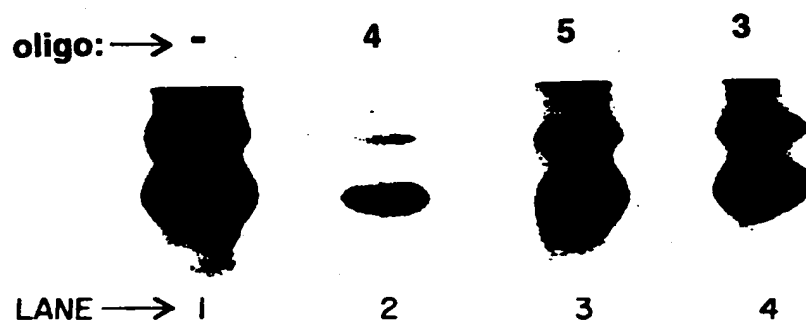
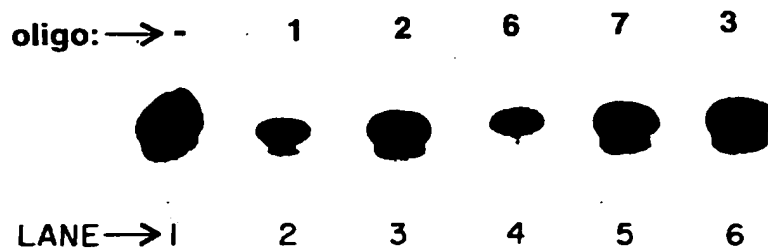
2/4

**FIG. 1B****FIG. 2A****FIG. 6A**

3/4

*FIG. 2B**FIG. 3**FIG. 4*

4/4

*FIG. 5**FIG. 6B**FIG. 7*

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/01008

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 C07H21/00 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANTISENSE RES. DEV. 4 (1994);279-84, XP002005503 TEMSAMANI, J. ET AL.: "Inhibition of in vitro transcription by oligodeoxynucleotides" see the whole document ---	1-26
A	CHEMICAL REVIEWS, vol. 90, no. 4, 1 June 1990, pages 543-584, XP000141412 UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" see the whole document -----	4,10,14, 18,22,26

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 June 1996

Date of mailing of the international search report

1 0. 07. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Andres, S